Docket No.: 62660

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
A. Domling

Application No.: 10/520,791 Confirmation No.: 3248

Filed: January 8, 2005 Art Unit: 1654

For: TUBULYSIN CONJUGATES Examiner: S.R. Gudibande

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.132

I, Alexander Domling, declare as follows:

1. I am the inventor on the above-identified patent application (referred to below as "the patent application"). I earned a Ph.D. degree in Chemistry from the Technical University in Munich in 1993. Subsequently, I was Vice-President Chemistry of Morphochem AG until 2003 and then in 2004 co-founded R&D Biopharmaceuticals GmbH. I am currently Associate Professor of Pharmacology at the University of Pittsburgh.

2. The following experimental work as detailed in paragraphs 3 through 5 below were conducted by me or persons working under my direction.

3. Preparation of tubulysin-PEG conjugate compounds

Tubulysin-PEG conjugate compounds of natural tubulysin A and polyethyleneglycol (PEG) were prepared as described in Example 1 of the patent application. The PEG reagents had molecular weights of 6kDa, 10kDa, 20 kDa, 35kDa and 40kDa as specified in Tables 1 and 3 below.

All tubulysin-PEG conjugate compounds were characterized by HPLC-MS studies.

4. Solubility tests

It was found that the prepared tubulysin A-PEG conjugate compounds exhibited an increase in solubility in Na phosphate buffer (10 mM) pH 5-7 and in Na phosphate buffer (10 mM) pH 8 between 10-40 fold in contrast to Tubulysin A alone. Results for tubulysin A-PEG conjugate compounds are set forth in Table 1 below. Results for natural tubulysin A (not conjugated) are set forth in Table 2 below.

Table 1: Thermodynamic solubility (25°C, mg/l) of Tubulysin A – PEG conjugated compounds in different buffers.

Solvent	Tubulysin A-PEG conjugate compound	Solubility
Na phosphate buffer (10 mM) pH 5-7	TubuA-Peg 8kDa	1.1 mg/ml
Na phosphate buffer (10 mM) pH 8	TubuA-Peg 8kDa	6.5 mg/ml
Na phosphate buffer (10 mM) pH 5-7	TubuA-Peg 35kDa	3.2 mg/ml
Na phosphate buffer (10 mM) pH 8	TubuA-Peg 35kDa	18.5 mg/ml
Na phosphate buffer (10 mM) pH 5-7	TubuA-Peg 40kDa	4.6 mg/ml
Na phosphate buffer (10 mM) pH 8	TubuA-Peg 40kDa	24.1 mg/ml

Table 2: Thermodynamic solubility (25°C, mg/l) of Tubulysin A (not conjugated) in different buffers.

Solvent	Solubility
Na phosphate buffer (10 mM) pH 5-7	0.1 mg/ml
Na phosphate buffer (10 mM) pH 8	0.6 mg/ml

5. Cell data

The tubulysin A-PEG conjugate compounds identified in Table 3 below were tested in an acid phosphatase assay for activity against human cancer cell lines of MCF7 and KB-V1 the table below. The protocol of the acid phosphatase assay was as described in Yang, T.T., Sinai, P., Kain, S.R. (1996) Anal. Biochem. 241: 103.

Table 3: IC50 values [ng/ml] of various Tubulysin A-PEG conjugate compounds against different cancer cell lines.

Tubulysin A-PEG conjugate compounds	MCF7 IC50 [ng/ml]	KB-V1 IC-50 [ng/ml]
TubuA-PEG ester 6kDa	9	8
TubuA-PEG ester 35kDa	18	19
TubuA-PEG ester 40kDa	24	28
TubuA-PEG amide 10kDa	13	16

TubuA-PEG amide 40kDa	27	33
TubuA-PEG phenol ester 10kDa	7	6
TubuA-PEG phenol ester 40kDa	19	34

8. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing therein.

Date: 11/30/06

Alexander Domling